Renewable and Sustainable Energy Reviews 27 (2013) 596–604

Contents lists available at ScienceDirect

Renewable and Sustainable Energy Reviews

journal homepage: www.elsevier.com/locate/rser

Algae biomass as an alternative substrate in biogas production technologies—Review

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A R T I C L E I N F O

Article history:
Received 15 April 2013
Received in revised form
9 July 2013
Accepted 14 July 2013
Available online 7 August 2013

Keywords:
Algae biomass
Biogas production
Methane fermentation
Microalgae
Macroalgae
Photobioreactors

A B S T R A C T

The use of algae as a potential substrate in biogas production processes has been discussed sporadically, therefore this manuscript provides an overview of reference data published so far on that matter. The goal of this review is to present possibilities of applying algae biomass for biogas production purposes and to determine the effectiveness of the fermentation process of algae belonging to various taxonomic groups, originating from various biocenoses and characterized by different morphology and properties. Finally, this work reports on methods and technological solutions for algae biomass production as well as impediments and opportunities stemming from algae biomass use in biogas production technologies.

Contents

1. Introduction

The development and wild-scale implementation of clean technologies for energy production is nowadays both a challenge to scientists and a priority to operators of energetic systems. Biomass of various origins, properties and energetic transformability is commonly believed to be one of the main sources of renewable energy [1–4]. But still, some reports undermine this common opinion. Fargione et al. [5] and Searchinger et al. [6] stated that the irrational management of resources of typical energetic crops might, in practice, lead to increasing quantities of greenhouse gases being emitted to the atmosphere. Some works suggest also that intensive exploitation of arable lands for the cultivation of crops dedicated for biofuels production may yield a negative impact on the global supply and prices of foods [7].

In view of the above, an urgent need emerges for alternative sources of biomass for energetic purposes that would be both economic and environment-friendly. Considering a very high photosynthetic effectiveness, a fast rate of biomass growth, resistance to

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http://dx.doi.org/10.1016/j.rser.2013.07.029

various types of contaminations, and possibility of land management that cannot be used for other purposes, algae appear as a competition to typical energetic crops [8–13].

Most of research works published so far have been focused on biodiesel production technologies based on lipids that are accumulated in large quantities in cells of algae [8,14]. In contrast, the use of microalgae and macroalgae as potential substrates in processes of biogas production has recently been addressed only sporadically. Available works analyze the course and effectiveness of the fermentation process with unicellular algae, e.g. *Scenedesmus sp*. [15], *Spirulina sp*. [16–18], *Euglena sp*. and *Chlorella vulgaris* [19], *Melosira sp*. and *Oscillatoria sp*. [20], or macrophytobenthos organisms, e.g. *Laminaria sp*., *Macrocystis sp*. [21], *Gracilaria ceae* [22], and *Ulva sp*. [23] used as substrates. While others confirm that algae of *Macrosystis pyrifera*, *Tetraselmis*, *Gracilaria tikvahiae*, *Hypnea sp*., and *Ulva sp*. species may prove efficient as organic substrates in methane fermentation processes [24,25].

The objective of this review article is to present the applicability of algae biomass for biogas production purposes and to determine the effectiveness of methane fermentation of algae from different taxonomic groups, various production systems, and characterized by different properties and physicochemical characteristics.

2. Sources of algae biomass

A key element which in many cases determines the cost-effectiveness of algae biomass utilization for biogas production purposes is the choice of their culture technologies [26]. Algae may be cultured with various methods, beginning from technologically-advanced solutions in which the process is thoroughly monitored and controlled, to less predictable techniques based on open tanks [27]. Algae biomass may as well be acquired for biogas production purposes from natural, eutrophicated and degraded water bodies [28].

The available reports on biogas production from the biomass of algae harvested from natural aquifers refer mainly to the use of blue–green algae originating from eutrophicated lakes of China [29,30,28] and macrophytobenthos obtained on a small-scale [31–33], or include theoretical considerations, estimates and calculations of the potentiality of such technological solutions [34–39]. The acquisition of algae from the natural environment is substantiated by the immense productive potential of this source of biomass that may reach hundreds tones a day [28]. Investigations conducted so far enable concluding that nowadays this type of substrate is increasingly often considered as a potential source of organic matter used in processes of biogas production [29,30,28].

Intensive bloomings of blue–green algae are an increasingly frequent phenomenon observed in many aquifers worldwide. They disturb the natural functioning of aquifers, undermine the development of tourism and recreation, and finally diminish the industrial applicability of algae themselves. In recent years, such a phenomenon has been systematically occurring in lakes Chaohu, Taihu or Dianchi in China [40]. For instance, Guo [41] describes this phenomenon in the lake Taihu, which is the third in size lake in China that provides drinking water to over two millions people. The bloomings of unicellular blue–green algae observed systematically since 2007 pose technological difficulties in the conditioning of water to be used for drinking. In turn, Zhong et al. [28] describe the possibility of applying the biomass of algae, mainly *Cyanoprokaryota*, from this aquifer in the process of methane fermentation.

The use of biomass of algae from natural aquifers, especially these located in the temperate climatic zone, involves many difficulties [42]. They may be linked with variable climatic conditions, which directly affect the quantity of biomass to be acquired, taxonomic structure of algae as well as characteristics and properties of organic substrate obtained in this way. In the winter period, owing to a reduced rate of vegetation, a very low concentration of algae biomass in natural water bodies and frequently-occurring ice cover, the acquisition of biomass is ineffective both from the technological and economic point of view.

For this reason, algae biomass to be used for energetic purposes is proliferated and cultured in installations exploited under controlled conditions. These may include open and closed installations of different designs [43–47]. The technological systems of this type eliminate or significantly reduce problems observed during algae harvest from natural water bodies [45]. The open systems include, among others, traditional ground or concrete ponds, circular ponds with mechanical stirring, race-track type ponds with a paddle wheel or cascade ponds [48,49]. In turn the closed systems are photobioreactors of different constructions. Unlike the open systems, they enable constant control over the intensity and time of exposure to light, temperature of culture medium, as well as reduce the risk of predators, parasites and competitive algae species access to tanks. The most commonly applied photobioreactors include: the sack system, tubular photobioreactors with horizontal or vertical flow or inclined under any angle, biocoil type reactors, and plate photobioreactors [43–45]. Photobioreactors are significantly more universal facilities that may be applied in different climatic zones. This allows the culture of selected algae species, e.g. with a high concentration of oil in biomass. Unfortunately, attempts of constructing such a system that would additionally be cost-effective have so far been unsuccessful. The construction of photobioreactors is costly as they require high exploitation inputs (lighting, supply of carbon dioxide) and pose difficulties in exploitation due to, e.g. overgrowing and reduced light penetration [50–53]. Literature data on the effectiveness of algae biomass production and biomass concentrations achieved in technological systems was collated in Table 1.

3. Limitations in algae use for biogas production processes

A reduced interest in algae biomass as an alternative source of biodegradable organic matter applied in biogas production systems is mainly due to difficulties with its use as a substrate. Thus far conducted investigations pinpoint several factors that curb the effectiveness of methane fermentation process and may effectively reduce or completely inhibit the mechanism of biogas production in technologies utilizing algae biomass [21,41,15,29,19,60]. These factors include: cell walls that are resistant to degradation under anaerobic conditions owing to the presence of cellulose or hemicellulose compounds, strains of algae producing substances and compounds toxic to anaerobic bacteria, and the unbeneficial C:N ratio in the biomass subjected to the fermentation processes [61,62].

Such technological difficulties were proved by Dębowski [63]. In his experiments, analyses were aimed at verifying or confirming a direct relationship between the course and technological effects of fermentation, and the taxon of microalgae applied in the process. To this end, pure cultures of microalgae from the *Chlorophyta*, *Cyanoprokaryota* and *Bacillariophyceae* phylum were proliferated under controlled conditions and their biomass was used in the fermentation process. The use of algae from the pure culture *Chlorophyta* division during methane fermentation enabled reaching an average biogas production yield at 396.21 ± 30.94 dm^3/kg o.d.m. at the reaction rate of $r = 54.28$ cm^3/d. Methane content of biogas accounted for 59.73 ± 2.43%. In the series where algae of the *Cyanoprokaryota* divisio served as the organic substrate, the yield of biogas production oscillated around 382.45 ± 9.24 dm^3/kg o.d.m., biogas was produced

Table 1
Comparison of systems for algae biomass production and culture.

Culture system	Biomass concentration	Production effectiveness	References
Open systems			
Race track-type ponds	0.35 g d.m./dm^3	11.0 g d.m./m^2 d	[54]
	0.25–1.0 g d.m./dm^3	10.0–20.0 g d.m./m^2 d	[55]
	–	21.0 g d.m./m^2 d	[56]
	1.8 g d.m./dm^3	11.4 g d.m./dm^2 d	[49]
Closed systems			
Tubular photobioreactors	1.5–1.7 g d.m./dm^3	15.0–20.0 g d.m./m^2 d	[55]
	–	30.0 g d.m./m^2 d	[56]
	1.02 g/dm^3	25.0 g d.m./m^2 d	[54]
Biofilm photobioreactor	89.6–103.2 g d.m./dm^3	0.65–0.77 g d.m./m^2 d	[54]
	70.0 g d.m./dm^3	–	[55]
Biocoil type reactor	–	2.0 g d.m./dm^3 d	[57]
Flat—plat photobioreactor	2.7 g d.m./dm^3	27.0 g d.m./m^2 d	[54]
Airlift	1.20 g d.m./dm^3	20.0 g/m^2 d	[58]
Alveolar	1.45 g d.m./dm^3	–	[59]

at the mean rate of $r=97.14$ cm^3/d, and the percentage content of methane in biogas accounted for 63.08 ± 3.10%. The poorest technological effects were noted in the series with a pure culture of algae of the *Bacillariophyceae* division used as the substrate. Therein, the effectiveness of biogas production was at 357.07 ± 2.20 dm^3/kg o.d.m., the mean rate of gaseous metabolites production was $r=51.06$ cm^3/d, whereas methane content reached 57.83 ± 3.09%. The fermentation process was difficult due to the biological decomposition of cell walls of algae of the class *Bacillariophyceae*.

The use of biomass of algae originating from eutrophicated surface waters may also pose exploitation and technological difficulties in biogas production systems. They are linked with varying quality of biomass originating from this source and with difficulties in assuring substrate supply at a stable level [46,42,64]. Such technological difficulties were described by Dębowski [63], who was investigating fermentation of microalgae biomass obtained from the Vistula Lagoon in different periods of the season. The study was aimed at establishing the effect of the taxonomic structure, characteristics and properties of algae biomass obtained in different periods of the vegetative season on the course of the methane fermentation process. Experiment I was focused on the analysis of methane fermentation of microalgae biomass obtained from the Vistula Lagoon. Determinations conducted at this stage of the study were aimed at establishing the effect of the taxonomic structure, characteristics and properties of algae biomass obtained in different periods of the vegetative season on the course of the methane fermentation process. The experiments proved a direct effect of the vegetative season the microalgae originate from and the resultant taxonomic structure of algae biomass on the course and final effects of anaerobic processes. The highest technological effects of the methane fermentation process were noted in the variants where model fermentation tanks were fed with algae biomass obtained since June till September when predominating were *Cyanoprokaryota* with subpredominating *Chlorophyta*. In this period, the yield of biogas production was in the range from 389.07 ± 8.21 dm^3/kg o.d.m. to 420.95 ± 0.95 dm^3/kg o.d.m., at the observed mean reaction rates were from $r=73.24$ cm^3/d to $r=87.66$ cm^3/d. A significantly higher effectiveness of biogas production was observed in the spring and autumn seasons when the predominating taxa were these of *Bacillariophyceae*. The total production of biogas in these cases was in the range from 316.99 ± 11.74 dm^3/g o.d.m. to 329.65 ± 25.85 dm^3/g o.d.m. The rate of biogas production and methane content were at $r=45.65$ cm^3/d to $r=53.73$ cm^3/d. In the period of *Cyanoprokaryota* prevalence, a significantly higher content of CH_4 was additionally observed in biogas, with its highest value of 71.37 ± 0.49% being noted during fermentation of algae collected in July.

The C:N ratio in substrates fed into fermentation tanks should range from 20:1 to 30:1. In most cases where use was made of algae biomass the value of C:N ratio was below 10, which had a direct impact on diminished effectiveness of fermentation processes [65]. A high content of protein in algae biomass may lead to enhanced production of free ammonia and volatile fatty acids that elicit toxic effects on microorganisms responsible for methane fermentation processes. In addition, sodium ions occurring in the biomass of algae originating from culture systems based on salt water may inhibit the process of methanogenesis. Nevertheless, some works report on the feasibility of adapting the microbiota of anaerobic sludge to effective fermentation of marine algae biomass [66,67].

The C:N ratio may be increased by, e.g. addition of biomass with a high concentration of organic carbon to a substrate mixture [68]. Yen and Brune [15] achieved a significant increase in methane fermentation as a result of co-fermentation of cellulose wastes and algae biomass. The rate of methane production increased to 1.17 dm^3/m^3 d in the variant where the ratio of organic wastes to algae biomass was 1:1, compared to the value of 0.57 dm^3/m^3 d in the variant with only algae subjected to the fermentation process.

Another study addressed the co-fermentation process of algae biomass and maize silage and Virginia mallow silage [63]. The objective of this type of experiments was to determine the effect of microalgae addition on the course of the fermentation process of a typical substrate used in agricultural biogas works. The use of a substrate composition based on algae biomass and typical energetic crops had an immediate effect on the improvement of the C/N ratio in the substrate fed to the exploited fermentation tanks. Experiments were conducted in continuous reactors and with varying ratios of organic dry matter originating from microalgae biomass to organic dry matter of terrestrial plants examined. The application of substrate compositions based on algae biomass and biomass of maize silage and Virginia mallow silage affected higher final effects of the methane fermentation process. In the case of algae biomass co-fermentation with maize silage, the production of biogas was observed to increase from 438.73 ± 25.21 dm^3/kg o.d.m. in the variant where only algae biomass was subject to fermentation to 628.00 ± 13.72 dm^3/kg o.d.m. in the variant where the ratio of o.d.m. of algae biomass/o.d.m. of maize silage was 40/60. The content of methane in biogas ranged from 65.12 ± 1.94% to 60.62 ± 4.13%. The greater contribution of maize silage affected a decrease in the effectiveness of the

fermentation process. Analogous dependencies were observed in the case of co-fermentation of algae biomass and Virginia mallow silage, however effects of this technological treatment were better [63].

Many investigations conducted so far with the use of algae biomass in fermentation processes were signalizing technological and technical difficulties in running the process that included, e.g. a complicated process of macroalgae biomass acquisition, high initial hydration of biomass, difficulties with its storage and high costs of its dehydration [23]. Main technology-related issues include the selection of an appropriate retention time, a load of organic matter fed into fermentation tanks, and methods of biomass conditioning and pre-treatment [30,59,60,69].

4. Potential benefits of using algae biomass for biogas production purposes

Despite some limitations of algae biomass use for biogas production processes, studies conducted so far enable acknowledging it as an alternative and prospective source of organic substrate. These organisms have many advantages over typical, higher energetic crops. The algae, mainly the marine ones, were shown to contain high quantities of polysaccharides and lipid substances and to be free of sparingly-degradable lignocellulose compounds [31]. They are also characterized by a higher rate of biomass growth, whilst the possibility of their harvest from natural aquifers make them a competition to crops dedicated for nutritional or feeding purposes [70,71].

The up-to-date studies confirm methane fermentation to be an effective technology for algae biomass conversion for energetic purposes. This was also confirmed in a work by Harun et al. [36], who carried out multi-variant calculation of the profitability of different methods of energetic exploitation of marine algae. In the first case it was assumed that all fractions would be utilized in methane fermentation process, whereas in the second case biogas was produced from a fraction of protein and lipids while carbohydrates were used for ethanol production. The third variant consisted in the use of proteins and saccharides, whereas lipids were used for biodiesel production. In the fourth solution, proteins were applied to produce methane. The theoretical model proved that the most advisable variant from the energetic and economic perspective was the one in which the entire biomass of algae was processed in the methane fermentation process [36]. Apart from high-energy biogas, an additional product of this process is post-fermentation sludge that may be used directly as a fertilizer for terrestrial plants or after simple treatment may be returned to the system of algae biomass culture as a medium component [72,73].

Wiley et al. [74] paid attention to the three components of the process train of cultivation, harvesting, and conversion into usable fuel. The paper compares various process train options and identifies knowledge gaps presently restricting the production of algal biodiesel and algae-derived biogas. Its analysis identifies energy-intensive processing and the inability to cultivate large quantities of lipid-rich algal biomass to be the major obstacles inhibiting algal biodiesel production. The summary of further states that anaerobic digestion of algal biomass requires fewer process train components and occurs regardless of lipid content. In either scenario, the use of wastewater effluent as a cultivation medium seems necessary to reduce greenhouse gas emissions and maximize water use efficiency. Furthermore, anaerobically digesting algal biomass generated from low-technology wastewater treatment processes represents an appropriate technology approach to algal biofuels that is poorly investigated. Coupling these processes can improve global health by improving sanitation, while providing a cleaner burning biogas alternative to indoor biomass cooking systems typical of less-developed areas [74].

5. The use of macroalgae in processes of methane fermentation

Investigations on the use of macroalgae of the brown algae division in processes of methane fermentation were conducted by Vergara-Fernàndez [31]. He was examining the possibility of applying to this end the biomass of *Macrocystis pyrifera* and *Durvillea antarctica* macroalgae and a substrate based on the mixture of these species. His study proved that for all substrates tested the yield of biogas production was comparable and reached 180.4 ± 1.5 dm^3/kg d.m. d. The use of the algae mixture had a direct impact on the fact that the yield of biogas production was lower and reached nearly 158.3 dm^3/kg d.m. d. The percentage content of methane in gaseous metabolites of anaerobic bacteria ranged from 60.0% to 70.0%, at the mean value approximating 65.0%, irrespective of algae species applied [31].

Singh and Gu [75] and Parmar et al. [76] were also analyzing the yield of biogas production with the use of microphytobenthos plants as an organic substrate. They achieved the highest technological effects during fermentation of *Laminaria digitata* brown algae belonging to the order *Laminariales*. In that case, methane production was high and reached 500 dm^3 CH_4/kg o.d.m. The use of *Macrocystis sp*. enabled achieving 390–410 dm^3 CH_4/kg o.d.m., whereas upon the use of *Gracilaria sp*. and *Laminaria sp*. methane production accounted for 280–400 dm^3 CH_4/kg o.d.m. and 260–280 dm^3 CH_4/kg o.d.m., respectively. The lowest final effects were noted in the fermentation process of algae of *Ulva sp*. belonging to the class of chlorophytes i.e. barely 200 dm^3 CH_4/kg d.m. [75,76]. A research by Dębowski et al. [47] proved that effects of the fermentation process of macroalgae originating from the Puck Bay, Poland, were directly dependent on the applied loading of anaerobic tanks with a feedstock of organic compounds. The highest methane production (240 dm^3 CH_4/kg o.d.m.) was noted in the loading range from 1.0 kg o.d.m./m^3 d to 2.0 kg o.d.m./m^3 d. The application of higher values of this technological parameter had a direct effect on methane production decline [47]. In turn, Yuan et al. [30] proved that methane production in the fermentation process of blue–green algae reached 189.89 dm^3 CH_4/kg o.d.m. Furthermore, Zeng et al. [29] were analyzing the course of methane fermentation of algae biomass with a prevailing species *Macrocystis sp*. in the process of co-fermentation with bovine liquid manure. The yield of methane fermentation in this experiment was 153.66 dm^3 CH_4/kg o.d.m. Other investigations examining the effectiveness of biogas production were carried out with, among others, macroalgae including *Laminaria sp*., *Macrocystis sp*. [21], *Gracilariaceae* [22], and *Ulva sp*. [23]. They corroborated the feasibility of using this type of organic substrate in methane fermentation processes.

Experimental works by Vergara-Fernàndez [31] demonstrated a high concentration of NH_3 at ca. 1.0% and of H_2S at 0.1% in biogas. The high concentration of NH_3 was proved to be due to a high concentration of nitrogen in marine algae used in the experiment, and reached 15.8 mg/g d.m. for *M. pyrifera* and 16.7 mg/g d.m. for *D. antarctica*.

In a study conducted by Grala et al. [33], the methane fermentation process was run with the biomass of macroalgae based on a mixture of filiform brown algae of the genus *Pilayella* (90% contribution) and *Ectocarpus* (8% contribution) and sporadically occurring green algae of the genus *Enteromarpha*. Before the anaerobic process, the substrate was subjected to enzymatic hydrolysis with a mixture of the following enzymes: Celluclast 1.5 L, Novozym 188 and Hemicellulase, and to the process of hydrothermal depolymerization run for 120 min at a temperature of 200 °C under the pressure of 17 Ba. The pre-treatment of the substrate resulted in biogas production accounting for 40.0 dm^3/kg substrate and 54.0 dm^3/kg substrate in the optimal variants.

Table 2
Effectiveness of biogas production with the use of macroalgae as a substrate in methane fermentation processes.

Macroalgae taxon	Quantity of biogas/methane	References
Macrocystis pyrifera	181.4 ± 52.3 dm^3 CH_4/kg d.m. d	[31]
M. pyrifera+Durvillea antarctica	164.2 ± 54.9 dm^3 CH_4/kg d.m. d	[31]
D. antarctica	179.3 ± 80.2 dm^3 CH_4/kg d.m. d	[31]
Laminaria sp.	260–280 dm^3/kg o.d.m.	[75,76]
Gracilaria sp.	280–400 dm^3/kg o.d.m.	[75,76]
Macrocystis	390–410 dm^3/kg o.d.m.	[75,76]
Laminaria digitata	500 dm^3/kg o.d.m.	[75,76]
Ulva sp.	200 dm^3/kg o.d.m.	[75,76]
Macrocystis sp.	189.9 dm^3 CH_4/kg o.d.m.	[30]
Ulva lactuca	157–271 dm^3 CH_4/kg o.d.m.	[23]
Pilayella+Ectocarpus+Enteromarpha	40.0–54.0 dm^3/kg 29.2–39.4 dm^3 CH_4/kg	[33]

The content of methane was at ca. 73.0%. The effectiveness of biogas production with the use of macroalgae as a substrate in methane fermentation processes was presented in Table 2.

6. The use of microalgae in processes of methane fermentation

The first trials of methane fermentation of microalgae biomass based on a mixed culture of *Chlorella sp*. and *Scenedesmus sp*. were conducted by Golueke et al. [77]. They additionally compared the effectiveness of the fermentation process of algae biomass and sewage sludge and demonstrated that the quantity of biogas per kg of organic dry matter and qualitative composition of gaseous metabolites of anaerobic bacteria were comparable for both analyzed substrates. In the case of sewage sludge the effectiveness of the fermentation process reached 1020 dm^3/kg o.d.m., whereas for algae biomass it was at 986 dm^3/kg o.d.m. The concentration of methane in biogas ranged from 61.0% to 63.0% [76].

Zamalloa et al. [78] were analyzing the possibility of fermenting *Scenedesmus obliquus* (*Chlorophyta*) algae commonly occurring in freshwaters, marine algae *Phaeodactylum tricornutum* of the division *Bacillariophyceae* and blue green algae species *Spirulina platensis*. After 30 days of incubation they achieved 210 ± 3.0 dm^3 CH_4/kg o.d.m. in the case of *S. obliquus* and 350 ± 3.0 dm^3 CH_4/kg o.d.m. in the variant with *P. tricornutum* biomass being fed to model fermentation tanks. Byr comparison, in the fermentation process of *S. platensis* blue–green algae the yield of methane production after 30 days of incubation reached 280 ± 0.8 dm^3 CH_4/kg o.d.m.

Investigations on the fermentation of various taxonomic groups of microalgae were also conducted by other researchers. For instance Mussgnug et al. [79] were testing six species of phytoplankton commonly occurring in freshwaters and saltwaters (*Chlamydomonas reinhardtii*, *Dunaliella salina* and *S. obliquus* belonging to the class *Chlorophyceae*, *Chlorella kessleri* belonging to the class *Trebouxiophyceae*, *Euglena gracilis* belonging to the class *Euglenoidea* and blue–green algae *Arthrospira platensis* from the class *Cyanophyceae*. Algae of the species *D. salina* and *A. platensis* are saltwater organisms, whereas the other species are freshwater algae.

The above authors proved that the potential of biogas production was directly determined by the species, whereas no correlation was found between the taxonomic groups and process effectiveness. The fermentation of *C. reinhardtii* algae from the division *Chlorophyta* resulted in biogas production at 587 ± 8.8 dm^3/kg o.d.m., whereas that of *D. salina* algae in biogas production at 505 ± 24.8 dm^3/kg o.d.m. The anaerobic processes applied to blue–green algae *A. platensis* and *E. gracilis* resulted in the production effectiveness of gaseous metabolites of fermentation bacteria at 481 ± 13.8 dm^3/kg o.d.m. and 485 ± 3.0 dm^3/kg o.d.m., respectively. In turn, biogas production from the biomass of *C. kessleri* and *S. obliquus* algae was the lowest and accounted for 335 ± 7.8 dm^3/kg o.d.m. and 287 ± 10.1 dm^3/kg o.d.m., respectively [79]. These authors postulated that biogas production was not dependent on the taxonomic group of algae and that the potential effectiveness of the fermentation process cannot be predicted based on the systematic classification. They have concluded that precise determination of the quantity and composition of biogas produced is feasible upon individual verification of experiments for each of the analyzed species. Finally, the susceptibility of individual species and taxonomic groups of algae in this respect is, probably, attributable to the structure of their cell walls.

Available literature works report on a correlation between the structure of cells of the analyzed microalgae and biomass susceptibility to degradation under anaerobic conditions and intensity of biogas production. All easily-biodegradable species of algae, that enabled achieving high technological effects, were characterized by a lack of the cell wall, as in the case of *D. salina* [60], or their cell wall did not contain sparingly-degradable cellulose and hemicellulose components and was made of protein substances, as in the case of *C. reinhardtii* [81], *A. platensis* [82] and *E. gracilis* [83]. Contrary to the aforementioned species, *C. kessleri* and *S. obliquus* are characterized by cell walls built of hemicellulose [84,85]. The cell wall of *S. obliquus* is described in literature as especially difficult to break owing to the presence of a sporopollenin biopolymer [86]. Even more complex is the silica structure of the cell wall of *Bacillariophyceae* [87].

Data achieved so far demonstrate that the presence and composition of the cell wall are the main reasons behind differences in algae biomass degradability under anaerobic conditions and effectiveness of biogas production determined in own studies. When selecting macroalgae for the methane fermentation process, preferred shall only be species devoid of the cell wall or with the cell wall free of hemicellulose compounds. However, even in that case it cannot be excluded that the algae without a rigid cell wall may be an inappropriate substrate for the effective fermentation process owing to the possibility of the presence of substances toxic to anaerobes [66,67,87].

De Schamphelaire and Verstraete [88] presented a research on the effectiveness of biogas production with mixed cultures of freshwater macroalgae. The application of the initial loading of batch fermenters at a level of 0.6 g o.d.m./dm^3 d resulted in methane production at 310 dm^3 CH_4/kg o.d.m. after 45 days of biomass retention in the technological system.

Samson and LeDuy [17] were fermenting biomass of blue–green algae species *Spirulina maxima* in a reactor exploited at the loading of 1.0 g o.d.m./dm^3 d and at biomass retention time of 33 days. Methane production in the mesophilic process accounted for 240 dm^3 CH_4/kg o.d.m. on average, at the observed effectiveness of organic biomass degradation ranging from 68.0% to 72.0%. Ras et al. [19] achieved the yield of biogas production at 150 dm^3 CH_4/kg o.d.m. and 240 dm^3 CH_4/kg o.d.m. after hydraulic retention

time of 16 and 28 days, respectively. The experiment was run with *C. vulgaris* biomass and the loading of exploited fermentation tanks was kept at 1.0 g COD/dm^3 d. The effectiveness of biomass conversion to gaseous metabolites of anaerobic bacteria fitted within the range of 29.0% to 49.0%.

Similar observations were made by Zamalloa et al. [78] who achieved the COD:N ratio at 10.3 ± 0.6 when examining the biomass of *S. platensis*. It was due to a very high content of proteins in the biomass, reaching 60%. In the case of two other analyzed species of algae, the ratio of carbon substances expressed by the COD value to nitrogen was comparable and reached 15.6 ± 7.4 for *S. obliquus* and 13.4 ± 3.4 for *P. tricornutum*.

7. Co-fermentation of algae biomass with typical energetic crops

According to Mata-Alvarez et al. [61], the application of a substrate mixture in the fermentation process may improve the effectiveness of methane fermentation process when compared to the processes conducted with individual substrates. This was also proved in our previous works [89,26]. This solution enables system supply with any microelements and nutrients necessary for the growth of anaerobic microflora [62]. According to the authors of this review as well as other scientists, the appropriate selection of co-substrate may also yield other benefits linked with technological, economic and environmental aspects of the methane fermentation process [62,32,68,69].

Considering the real concentration of algae in natural water bodies, it ought to be stated that organic substrate of this type may be treated as one of the components of a substrate mixture fed into fermentation systems exploited in the technical scale [29,30]. This may also improve the C:N ratio in the organic substrate dosed into anaerobic reactors.

Mussgnug et al. [79] demonstrated that the fermentation of maize silage and macroalgae under the same technological conditions enabled achieving a higher by 11.0% effectiveness of methane fermentation in the case of *C. reinhardtii*. In the study conducted by Zhong et al. [28], the applied substrates were algae from the Meiliang Bay at the Lake Taihu in China and maize silage. In the case of algae, prevailing were the blue–green algae of the genus *Microcystis sp*. (99%). Experiments were carried out with reactors having the total volume of 150 cm^3, fed with 2.0 g o.d.m. originating from biomass of algae and maize. These two basic substrates were dosed in at different proportions, which had a direct impact on the C:N ratio in the consecutive variants of the experiment, i.e. 71:1, 25:1, 20:1, 16:1, and 6:1. Based on the total volume of biogas produced within 30 days of incubation it may be concluded that the highest technological effects were achieved in the variants with C:N ratio ranging from 16:1 to 25:1, thus assuring the value of this parameter in the range from 922 cm^3 to 1184 cm^3. The highest percentage content of methane in biogas (54.90%) was observed in the variant with C:N at 20. In the other cases, methane concentration oscillated around 51.0% [28].

The earlier own investigations [90,91] proved the positive effect of addition of macroalgae biomass from the Puck Bay on the effectiveness of methane fermentation of hay silage and maize silage. In the best variants, methane fermentation reached 373.1 m^3 CH_4/Mg o.d.m. under static conditions and 386.8 dm^3 CH_4/kg o.d.m. during experiments in flow reactors in the case of testing algae biomass and maize silage [91]. A lower process effectiveness was determined during the fermentation of aquatic vegetation biomass and hay silage. In respirometric experiments the production of methane accounted for 354.7 dm^3 CH_4/kg o.d.m., whereas in the continuous fermentation tanks for 359.0 dm^3 CH_4/kg o.d.m. [90].

The increased effectiveness of the fermentation process during co-fermentation of algae biomass and other organic substrates is ascribed to its synergistic effects occurring during anaerobic degradation of the substrate mixture. The biomass of algae serves as a source of nitrogen and microelements indispensable for the appropriate growth of anaerobic microorganisms. Mata-Alvarez et al. [61] proved that the introduction of a few appropriately selected substrates to a fermentation bioreactor improved the final effects of the anaerobic process. Matsui and Koike [32] were exploiting a pilot technological system where the main organic substrate were macroalgae of the genus *Laminaria sp*. and *Ulva sp*. mixed with other organic wastes. They proved that reaching stable conditions of the anaerobic installation work was feasible upon appropriate selection of the proportions of aquatic vegetation and the analyzed co-substrate. The effectiveness of biogas production with the use of microalgae as a substrate in methane fermentation processes was presented in Table 3.

8. Summary

Algae may serve as potential sources of many types of biofuels, these including: biogas produced in processes of anaerobic degradation of biomass, biodiesel produced from lipids accumulated in cells of algae, alcohol, hydrogen from photobiological transformations or algae biomass that may be used for direct combustion. Many scientists claim that the use of methane fermentation is a more prospective and the most effective method for the energetic exploitation of algae biomass. Sialve et al. [34] stated that the application of methane fermentation under appropriate technological conditions as the primary method of algae biomass conversion assured a higher economic effect compared to the integrated system of lipids extraction and anaerobic processing of post-extraction residues. Results described by other authors suggest that the balance of unit operations run during methane fermentation process is the most effective both from the perspective of economic analysis and emission of contaminants to components of the environment [92]. Results of investigations prove that methane fermentation may be the most practical means of algae biomass conversion into energy. However, Börjesson and Berglund [4] noticed that energetic inputs and consequences to the environment were highly diversified depending on the type of technological solutions of the methane fermentation process. For this reason, in each case the complete and objective evaluation requires the environmental life cycle assessment (LCA).

Production systems of algae biomass to be used for biogas production ought to integrated with technological installations of wastewater treatment. Solutions of this type are more substantiated from both the economic and technological standpoint as they ensure the simultaneous removal of chemical and biological contaminants from wastewaters and production of biomass to be converted into biogas. Algae assimilate considerable quantities of biogenes contained in wastewaters because they need high quantities of nitrogen and phosphorus for the systemic synthesis of proteins, the concentration of which in dry matter ranges from 20% to 60%, depending on species. The application of wastewaters as a culture medium contributes directly to reduction of costs incurred on the supply of water and nutrients necessary for the effective growth of algae biomass. In addition, a high CO_2 concentration in wastewaters intensifies the rate of algae biomass growth, which has a direct impact on the effectiveness of contaminants removal. In systems based on salt waters, the use of wastewaters enables additionally the equilibration of the molar ratio of carbon, nitrogen and phosphorus (C:N:P = 106:16:1), the so-called Redfield′s ratio.

Table 3
Effectiveness of biogas production with the use of microalgae as a substrate in methane fermentation processes.

Microalgae taxon	Quantity of biogas/methane	References
Scenedesmus sp.+Chlorella sp.	986 dm^3/kg o.d.m. 180 ± 8 dm^3/dm^3 d	[77]
Scenedesmus sp+Chlorella sp.	573 ± 28 cm^3/dm^3 d 818 ± 96 cm^3/dm^3 d	[15]
Scenedesmus obliquus	210 ± 3.0 dm^3 CH_4/kg o.d.m.	[78]
Phaeodactylum tricornutum	350 ± 3.0 dm^3 CH_4/kg o.d.m.	[78]
Spirulina platensis	280 ± 0.8 dm^3 CH_4/kg o.d.m.	[78]
Chlamydomonas reinhardtii	587 ± 8.8 dm^3/kg o.d.m.	[79]
Dunaliella salina	505 ± 24.8 dm^3/kg o.d.m.	[79]
S. obliquus	287 ± 10.1 dm^3/kg o.d.m.	[79]
Chlorella kessleri	335 ± 7.8 dm^3/kg o.d.m.	[79]
Euglena gracilis	485 ± 3.0 dm^3/kg o.d.m.	[79]
Arthrospira platensis	481 ± 13.8 dm^3/kg o.d.m.	[79]
Spirulina maxima	240 dm^3 CH_4/kg o.d.m.	[18]
Chlorella vulgaris	150 dm^3 CH_4/kg o.d.m. 240 dm^3 CH_4/kg o.d.m.	[19]

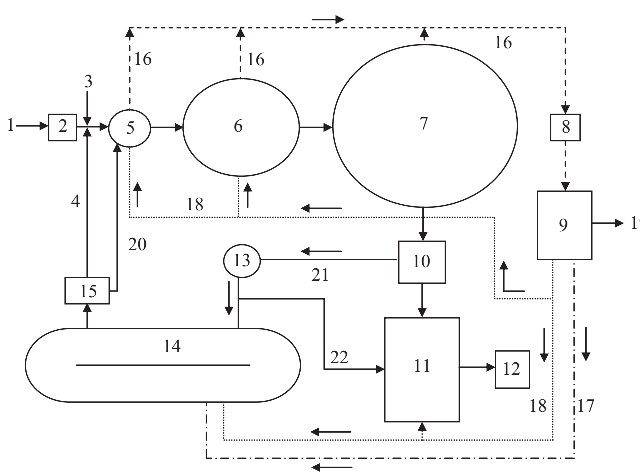


Fig. 1. Scheme of a technological system for co-fermentation of algae biomass and maize silage (1—maize silage, 2—feeding screw with shredder, 3—algae biomass originating from natural aquifers, 4—algae biomass originating from PBR, 5—hydrolyzing unit, 6—exact fermentation tank, 7—post-fermentation tank, 8—biogas purification system, 9—co-generating unit, 10—module for sludge concentration and dehydration, 11—sludge storage and drying, 12—storage of dried sludge (pellet production), 13—dissolved air flotation (DAF) unit, 14—system for proliferation of PBR algae biomass, 15—module for separation and concentration of algae biomass, 16—biogas installation, 17—CO_2 supply installation, 18—heat exchangers installation, 19—electric energy for own purposes of the installation and directed to the network, 20—directed liquid, 21—eluate, medium feeding the PBR, 22—post-flotation precipitate).

The second research direction that ought to be pursued in the perspective of algae biomass application for biogas production includes experiments aimed at developing methodology of the methane fermentation process. Herein, main issues include the selection of an appropriate retention time, load of organic matter fed to fermentation bioreactors, as well as methods of biomass conditioning and pre-treatment. Similar conclusions were drawn by authors of this manuscript when summarizing own studies conducted for many years and in most cases with the use of own technological solutions for the acquisition and separation of this substrate, storage and proliferation of algae biomass and for conducting the fermentation process.

Experimental and implementation works should focus on technologies for pre-treatment and conditioning of algae biomass to assure cell wall damage and provide organic matter to anaerobic microorganisms, with the latter two having a direct impact on methane fermentation process effects. Attention should be given to methods based on ultrasound or electrokinetic disintegration, hydrothermal depolymerization or to biochemical methods based on the use of enzymatic biopreparations. Another challenge to scientists is the elaboration of substrate mixtures based on algae biomass that would assure the appropriate C:N ratio for methane fermentation. It is of outmost significance especially for the biomass of algae harvested from natural aquifers which additionally poses the risk of high variability of taxonomic composition.

Ultimately, it is also feasible to design and construct closed systems in which biogas may be produced in the fermentation process of organic substrates with the use of algae biomass. Next, the eluate from dehydration of post-fermentation bulk and CO_2 from biogas combustion may be streamed to modules for algae biomass proliferation and culture. The algae biomass thus produced will constitute an additional source of organic substrate in the installation for biogas production. A scheme of a technological solution was presented in Fig. 1.

Acknowledgments

This work has been financed by The Strategic Program of the National (Polish) Centre for Research and Development (NCBiR):"-Advanced technologies for energy generation. Task IV: Elaboration of integrated technologies for the production of fuels and energy from biomass, agricultural waste and other waste materials".

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